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Examples

Materials

Primers/Scorpions primers:

B2098-BRCA Scorpions: FAM-CGCACGATGTAGCACATCAGAAGCGTGCG-MR-HEG-
TTGGAGATTTTGTCACTTCCACTCTCAAA (SEQ ID NO: 1)

Underlined regions are the hairpin forming parts, FAM is the fluorescein dye, MR is a non-fluorogenic fluorophore attached to a uracil, HEG is the replication blocking hexethylene glycol monomer. The probe matches the "C-variant" of the BRCA2 polymorphism and mismatches the "A-variant".

R-186-98: untailed equivalent of B2098:TTGGAGATTTTGTCACTTCCACTCTCAAA
(SEQ ID NO: 2)

R187-98: opposing primer to the R186-98 and the equivalent Scorpions.

Z3702: the probe segment of the Scorpions B2098:

FAM-CGCACGATGTAGCACATCAGAAGCGTGCG-MR (SEQ ID NO: 3)

Template DNA: previously genotyped DNA prepared by proteinase K and phenol/chloroform extraction was used at 50ng per 50µl reaction. Genotypes were typically one homozygous A/A, one homozygous C/C and one heterozygote (A/C).

Buffer (1x): 10 mM Tris-HCl (pH 8.3), 1.2 mM or 3.5 mM MgCl₂, 50 mM KCl, dNTPs (each at 100 µM), gelatin at 0.01% (w/v).

Enzyme: AmpliTaq Gold (Perkin-Elmer/ABI) was included in the reaction mix at 2units/50µl reaction.

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Examples 7 and 8

Random coil embodiment and bimolecular embodiment

Scorpion B2731:

fam-AGGTAGTGCAGAGAGTG-mr-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC (SEQ ID
NO: 4)

Scorpion B4249 (no quencher on same molecule)

09974870-101201

fam-AGGTAGTGCAGAGAGTG-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC (SEQ ID NO: 5)

Quencher oligonucleotide (complement of the tail of B4249):

CACTCTCTGCACTACCT-mr (SEQ ID NO: 6)

ARMS primer R284-97: TTCGGGGCTCCACACGGCGACTCTCAAC (SEQ ID NO: 7)

ARMS primer R283-97: TTCGGGGCTCCACACGGCGACTCTCAAG (SEQ ID NO: 8)

Target is the H63D polymorphism of the human hereditary haemochromatosis gene (H/H), B2731 and B4249 are "common" primers to oppose the ARMS primers R283-97, R283-97. Cycling conditions and reaction composition as above. Primers (including *Scorpion* primers) were used at 500nM concentration.

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Example 9

No quencher embodiment

Scorpion B4249 (no quencher)

fam-AGGTAGTGCAGAGAGTG-h-GAGCCTCAACATCCTGCTCCCCTCCTACTAC (SEQ ID NO: 5)

ARMS primer R284-97

TTCGGGGCTCCACACGGCGACTCTCAAC (SEQ ID NO: 7)

09974870-10101